effect can be masked by the autocrine production of SCF.

Finally, we evaluated the effects of two KIT inhibitors, imatinib (Carvajal *et al.*, 2011) and sorafenib (Heinrich *et al.*, 2012) on the signaling pathways activated in KIT-N505I expressing cells. Sorafenib, and imatinib to a lesser extent, inhibited SCF-induced KIT autophosphorylation as well as ERK and AKT phosphorylations in these cells (Figure 2d). Therefore, the KIT-N505I mutation did not abolish the sensitivity of KIT to these inhibitors.

To conclude, we described a new KIT-N505I mutation in an acral lentiginous melanoma that confers the receptor an increased basal activity, associated with exacerbated signaling properties. The respective roles of mutation and overexpression of KIT in the acquisition of tumorigenic properties by these melanoma cells remain to be elucidated. Nevertheless, patients with melanoma bearing the KIT-N505I mutation might be eligible for treatment with imatinib or sorafenib.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Prognostic Impact of p62 Expression in Cutaneous Malignant Melanoma

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TO THE EDITOR

Accumulating evidence suggests autophagy, the principle catabolic process for lysosomal degradation of surplus macromolecules (Roy and Debnath, 2010), is fundamental to tumorigenesis. Impaired autophagy results in accumulation of cellular breakdown products, increased oxidative stress and neoplastic transformation (Mathew *et al.*, 2009); whilst efficient autophagy facilitates metastatic tumor survival through sustained metabolic activity (Roy and Debnath, 2010). Consequently, the

Abbreviations: AJCC, American Joint Committee on Cancer; DFS, disease-free survival; MSM, melanoma-specific mortality

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currently accepted view is that autophagy suppresses growth early in tumor development but promotes tumor survival at later stages (Garber, 2011).

p62 (also known as sequestosome-1/SQSTM1) is a multidomain adaptor protein transporting ubiquitinated proteins during autophagy (Moscat and Diaz-Meco, 2009); as such, p62 plays a role in selective autophagic degradation of a number of substrates (Ichimura and Komatsu, 2010; Komatsu and Ichimura, 2010). Normally, p62 is broken down along with its cargo within the autophagolysosome. However, impaired autophagy is accompanied by p62 accumulation, resulting in large p62/ubiquitinated protein aggregates (Komatsu and Ichimura, 2010); a process thought to be a key factor in tumorigenesis (Moscat and Diaz-Meco, 2009).

The present biomarker discovery study aimed to define immunohistochemical p62 expression as a prognostic biomarker in a retrospective cohort comprising 29 melanocytic nevi and 121 primary cutaneous melanomas (Supplementary Methods online and Supplementary Table S1 online). In keeping with the ''autophagy paradox,'' we hypothesized p62 levels would be elevated in melanoma compared to nevi, with the highest levels detected in early stage disease where conditions represent a pro-tumorigenic environment.

Comparison of median p62 expression levels between nevi and all melanomas revealed a significant increase in expression levels in melanoma (14.82%) compared to nevi (0.51%; Mann–Whitney *U*, $P<0.0001$) (Figure 1a), and a stepwise increase in median p62 expression levels with localized tumor development (0.51% in nevi to 44.5% in AJCC (American Joint Committee on Cancer) II disease, Mann–Whitney $P<0.0001$) (Figure 1b). A relative decrease in median p62 expression (to 10.25%) was then observed in primary tumors from patients with metastatic stage III/IV disease (stage II vs. stage III/IV Mann–Whitney $P=0.005$) (Figure 1b). This biphasic expression in primary melanomas thus mirrors the ''autophagy paradox''.

To determine the efficacy of p62 expression levels at identifying high-risk tumors at diagnosis, comparison of localized and metastatic disease (eventual AJCC stages I/II vs. stages III/IV) revealed significantly lower median p62 expression in the metastatic cohort (10.25 vs. 26.94% in AJCC I/II; Mann–Whitney, $P=0.016$). p62 levels were visibly bimodal, with a Wilcoxon signed-rank test confirming that 20% expression was an appropriate cut-point for undertaking survival curve analysis, and as such statistical modeling was based on tumor p62 expression above (''high p62'') or below 20% (''low p62'').

Univariate analysis in all tumors was used to assess disease outcome over a 7-year follow-up period and revealed a modest, yet statistically significant, reduction in disease-free survival (DFS) in patients with ''low p62'' tumors (40.9% of patients in the ''low p62'' group developed metastases compared to 21.8% in ''high p62'' tumors (log-rank (Mantel–Cox) test $P=0.03$, hazard ratio (HR) 1.66 (95% confidence interval (CI) 1.03–2.69))) (Figure 2a).

Comparable univariate analysis of melanoma-specific mortality (MSM) in the whole melanoma cohort revealed a nonsignificant trend towards an increased MSM in patients with ''low p62'' tumors (MSM 24.24% ''low p62'' tumors, vs. 14.55% in ''high p62'' tumors (log-rank (Mantel–Cox) test $P=0.18$, HR 1.5 (95% CI 0.83–2.74))). Univariate analysis of other pre-hypothesized risk factors for disease progression were calculated (Supplementary Methods online and Supplementary Table S2 online); as expected there was a significant increased risk of metastases with increasing Breslow depth and tumor ulceration in line with AJCC staging criteria (Balch *et al.,* 2009).

Univariate analysis of tumors pre-stratified to AJCC stage II at diagnosis revealed low p62 expression levels that were associated with a trend for worse DFS; with 67.74% of patients in the ''low p62'' cohort developing a metastasis within 7 years compared to only 39.13% in the ''high p62'' group (log-rank (Mantel–Cox) test $P=0.06$, HR 1.7 (95% CI 0.97–2.96)) (Figure 2b). A similar trend was seen in AJCC stage I disease, (log-rank (Mantel–Cox) test $P=0.38$, HR 1.53 (95% CI 0.58–4.09)).

MSM followed an identical trend with ''low p62'' AJCC stage II tumors resulting in a higher mortality rate compared to ''high p62'' AJCC II tumors (MSM 35.48 vs. 21.74% respectively (log-rank (Mantel–Cox) test $P=0.27$, HR 1.51 (95% CI 0.72–3.2))).

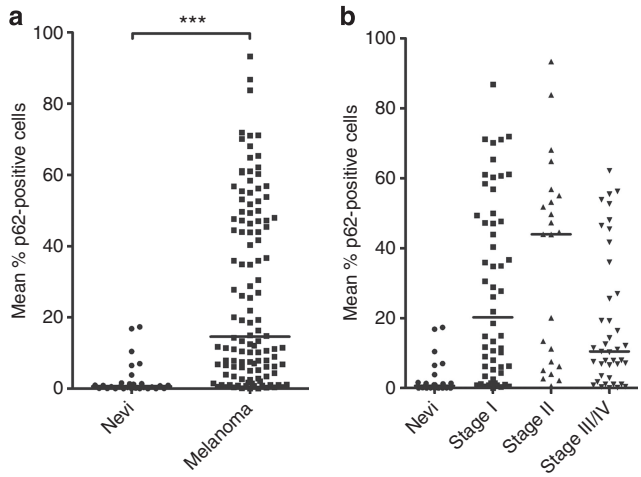

Figure 1. p62 expression in melanoma is consistent with the ''autophagy paradox'' in cancer. (**a**) Median p62 expression levels were significantly higher in melanoma compared to benign melanocytic nevi (Mann–Whitney *U*, $P<0.0001$). Horizontal lines represent median expression levels. (**b**) Median p62 expression levels increased between benign nevi and localized melanoma (eventual AJCC stages I and II), but revealed a relative fall in metastatic disease (eventual AJCC stages III and IV) (Kruskal–Wallis $P<0.0001$). Horizontal lines represent median expression levels. AJCC, American Joint Committee on Cancer.

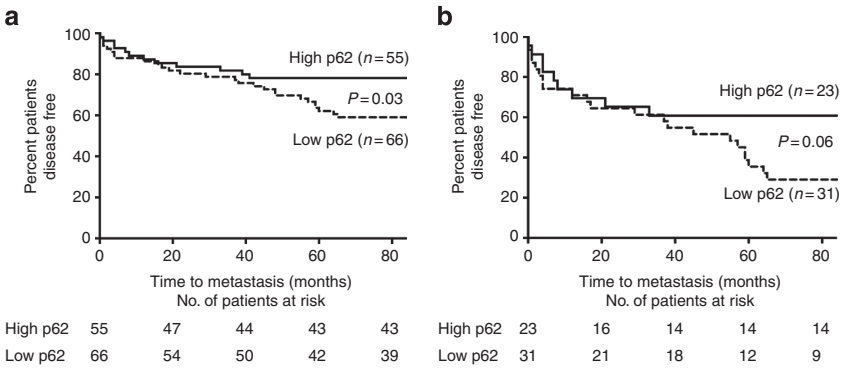

Figure 2. p62 as a potential prognostic biomarker in melanoma. (**a**) Univariate analysis of p62 expression revealed a significantly increased risk of metastasis in tumors expressing <20% p62 compared to tumors expressing >20% p62 (log-rank (Mantel–Cox) test $P=0.03$, HR 1.66 (95% CI 1.03–2.69)). (**b**) Analysis of p62 expression levels after initial stratification by AJCC stage of disease reveals a suggestive increased risk of metastasis in AJCC stage II primary tumors with p62 expression <20% compared to tumors expressing >20% p62 (log-rank (Mantel–Cox) test $P=0.06$, HR 1.70 (95% CI 0.97–2.96)). The number of patients remaining at risk at 20-month intervals is stated below the *x* axis. AJCC, American Joint Committee on Cancer; CI, confidence interval; HR, hazard ratio.

Comparison of mean p62 expression revealed no association with Breslow depth or tumor ulceration (Supplementary Figure S1 online) and Cox proportional hazards analysis undertaken to assess whether the predictive effects of p62 may be partially attributable to covariates (Supplementary Methods online and Supplementary Table S3 online) further supported p62 expression levels as an independent stratifying prognostic variable ($P<9E-7$), representing biologically distinct processes to those included in AJCC staging alone.

As a marker of autophagic activity, the p62 status of the current melanoma cohort fits with the ''autophagy paradox''; with the highest levels of p62 expression found in early, localized disease (AJCC stages I and II) in keeping with pro-tumorigenic dysfunctional autophagy. However, tumor cells with increased levels of autophagic activity (either through reactivation or retention of this function following tumorigenesis) are more likely to metastasize by harnessing pro-survival autophagy.

Independently of its role in autophagy, p62 expression may be regulated by other signaling mechanisms including via NF-κB activation and interaction with TRAF6 and caspase-8 (Mathew *et al.*, 2009; Komatsu and Ichimura, 2010). Therefore, the differential expression of p62 within different AJCC stages of melanoma cannot be assumed to result entirely from impaired autophagic activity. Nevertheless, results from the present study add to the growing body of evidence supporting the introduction of autophagy inhibitors to chemotherapeutic regimes in melanoma, stage I trials of which are currently underway (Komatsu *et al.*, 2007), for which p62 expression will likely provide a useful stratification criterion. Crucially however, p62 represents a potential candidate biomarker that may provide additional prognostic information to AJCC disease stage. Further validation in an independent retrospective and ongoing prospective cohort will determine the prognostic significance and effect size of p62 and its application as a biomarker for refining personalized therapies, ultimately translating into improved clinical outcome for melanoma patients.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

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